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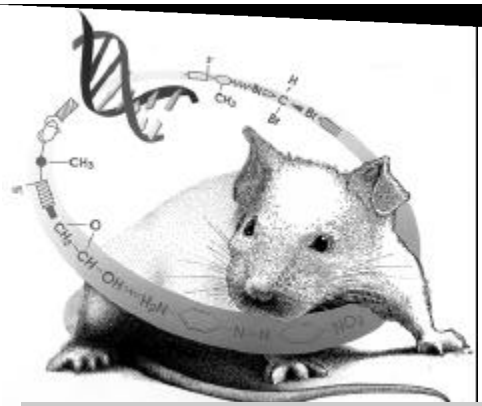


The Tg.AC Workgroup Newsletter

The Tg.AC Workgroup Newsletter is published by The Department of Toxicology and Safety Assessment, Boehringer Ingelheim Pharmaceuticals, Inc. as a means of communication for the HESI's Alternatives to Carcinogenicity Testing Committee.

Letter and article submissions are welcome. Persons interested in contributing to the newsletter should contact:

Sylvia M. Furst, Ph.D.
Boehringer Ingelheim Pharmaceuticals, Inc.
Research and Development Center
900 Ridgebury Rd.
P.O. Box 368
Ridgefield, CT 06877-0368

Tel: 203-791-6112
Fax: 203-789-5797
sfurst@rdg.boehringer-ingelheim.com

On the Inside:

Transgenic Models at SOT 1999

At the Society of Toxicology 38th Annual Meeting held in New Orleans on March 14-18 of this year, considerable attention was given to the application of Transgenic models in Toxicology. The program included a Continuing Education Course given by Dr. R. Cannon, Dr. M. Eddy, NIEHS and Dr. T. Goldsworthy, ILS, two Poster Discussion Sessions entitled "Transgenic Animals: Carcinogenicity Testing and Mechanisms" and "Alternative Models for Mutagenicity and Carcinogenicity Testing", in addition to a Roundtable Session on "A Partnership Approach to the Evaluation of Alternative Models for Carcinogenicity Testing" chaired by Dr. J. MacDonald, Schering-Plough and Dr. D. Robinson, ILSI. Much interest was shown in all of these sessions listed above with full capacity seating. The continuing education course familiarized the participants with the experimental transgenic models and their relevance for use in toxicology. Molecular aspects of transgene insertion, structure and expression, specific examples of transgenic models with toxicological applications as well as the current status of the evaluation of the models for safety assessment of drugs and chemicals was also presented. The Roundtable Session described the scientific and regulatory basis for the collaborative research effort on alternative models and provided perspectives by some of the participating government, industry and academic scientists on the models. Some interesting questions arose from these sessions, examples being; Are the transgenic models better than the traditional 2 year carcinogenicity studies and should we be comparing the data obtained from these studies to the bioassays as a gold standard? Are the models predictive for the bioassays or for human data? How do we explain responses which occur via mechanisms other than ras activation or loss of the p53 allele? How do we control/deal with the intrinsic instability of the transgenic model such as the Tg.AC? Given the existing data, how would one start over? How do we extrapolate the data obtained from transgenic models to humans? How does one address the issue of nongenotoxic compounds not detected due to site specific, species specific, or sex specific differences observed with these compounds in the bioassay? How do we select an MTD and determine dose levels in the 26 weeks studies with these models? In time these questions will be answered with continuing research and the collaborative effort to be addressed in the ACT Committee data evaluation workshop in the year 2000.

HESI Alternatives to Carcinogenicity Testing (ACT)
Minutes from the February 4, 1999 Conference Call
Update on Tg.AC Testing

The objective of the conference call was to obtain an update on the studies currently being performed or planned by members of the Tg.AC Working Group.

Each member reviewed the status of their studies including dates of initiation and completion for both the dose range-finding studies and the 6-month studies.

Dr. Tennant requested that any individual animal data that is available be posted on the NTP Website. This would enable statisticians access to the data for the evaluation of possible alternative statistical analysis methods. Dr. Tennant stressed that input is needed regarding statistical evaluation of the data obtained from the Tg.AC studies (eg. multiplicity, time to tumor). It was agreed that data should also be sent to members of the Statistics Subcommittee. The statisticians will be included on the distribution list for any summary reports.

Dr. Warner (Bristol Myers Squibb) inquired about the type of statistics which should be used to evaluate the Pathology results of the studies. Dr. Stoll indicated that BIPI does not use the Peto analysis in their statistical evaluations. Dr. Tennant will arrange to distribute an article regarding this issue to all members.

Dr. Robinson indicated that the contract for establishing a database has been initiated. Funding is available for Phase I and II (building structure of database and entering data). Dr. Robinson will check on the availability of funding for Phase III.

Dr. Stoll indicated to all members that hemizygous Tg.AC animals are to be used on all studies. As a result of the Quality Control program now in place at Taconic, to ensure animals are responders, problems with the responsiveness of the hemizygous Tg.AC mouse are no longer anticipated, based on data derived from the new colony.

Availability of the rasH2 mouse will not occur in 1999. According to Sam Phelan, Taconic has been granted an exclusive sublicense from DuPont to produce and distribute the rasH2 mouse developed by Dr. Tatsuji Nomura of Central Institute for Experimental Animals (CIEA) of Japan. Taconic will produce and distribute the animals worldwide except in Japan, where the CIEA will distribute the animals. It will take approximately one year before production levels are reached.

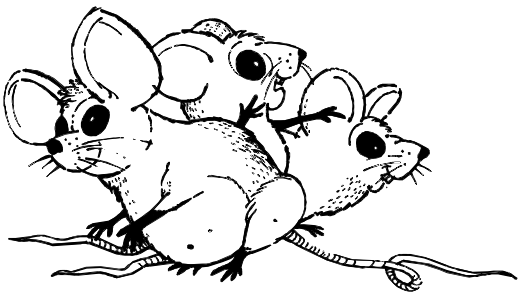
All agreed that data from completed dose range-finding and 6-month studies would be submitted for distribution to the Tg.AC Working Group members. This would include a summary report for completed studies including a proposed design of the subsequently planned 6-month studies.

The members have chosen September 16, 1999 for a Tg.AC Working Group meeting to discuss data from completed studies. The location of the meeting will be in Washingtion, DC at the ILSI offices. The Statistics Subcommittee would be invited to the meeting.

Assessment of a Rapid Cancer Bioassy Using the p53 Deficient Tg.AC (v-Ha-ras) Mouse Model

J. E. French, G. Lacks, G. Moser, T. L. Goldsworthy, J. W. Spalding and R W. Rennant. NIEHS and ILS, RTP, NC, USA

Genetically altered mouse models for toxicology and carcinogenesis experiments may provide a mechanistic basis for understanding carcinogen induced neoplasia. We conducted rapid cancer bioassays (26 weeks exposure) in this mouse model using benzene (200 µL, 2x/wk; topical), p-cresidine (0.5%, oral, diet), and TPA (1.5 µg, 3x/wk, topical) for comparison to previously conducted cancer bioassays in either FVB/N-Tg.AC(v-Ha-ras) or heterozygous C57BL/6-*Trp*53 mice. Mortality was greatest in p-cresidine treated mice due to rapid onset of urinary bladder masses. Mortality was also observed at lower levels in both untreated and treated dose groups due to the rapid onset of sporadic tumors or treatment related tumors. Clinical observations indicated that both benzene and TPA induced papillomas with an expected incidence and multiplicity, but benzene mice developed more test site skin and other malignancies than TPA treated mice relative to the controls. p-Cresidine treated transgenic mice (but not the nontransgenic control FVB/N mice) rapidly developed urinary bladder masses (100% males and 70% females). p-Cresidine induced body weight depression was similar in both transgenic and nontransgenic mice. Overall, these results indicate that carcinogen induction of the v-Ha-ras transgene under p53 deficient conditions may allow a rapid detection of carcinogen induced tissue specific malignancy. Both ras and p53 mutations are observed in many human cancers and a mechanistic understanding of the other critical genetic events occurring in these neoplasia may help in defining carcinogen specific mechanisms of cancer induction.



Articles of Interest

Tober KL, Cannon RE, Spalding JW, Oberyszyn TM, Parrett ML, Rackoff AI, Oberyszyn AS, Tennant RW and Robertson FM. (1998) Comparative expression of novel vascular endothelial growth factor/vascular permeability factor transcripts in skin, papillomas, and carcinomas of v-Ha-ras Tg.AC transgenic mice and FVB/N mice. Biochemical & Biophysical Research Communications 247(3): 644-53.

Trempus CS, Ward S, Farris G, Malarkey D, Faircloth RS, Cannon RE and Mahler JF. (1998) Association of v-Ha-ras transgene expression with development of erythroleukemia in Tg.AC transgenic mice. American Journal of Pathology 153(1): 247-54.

Cannon RE, Spalding JW, Virgil KM, Faircloth RS, Humble MC, Lacks GD and Tennant RW. (1998) Induction of transgene expression in Tg.AC (v-Ha-ras) transgenic mice concomitant with DNA hypomethylation. Molecular Carcinogenesis 21(4): 244-50.

Evaluation of the Tg.AC and p53 (het) Mouse as Potential Models for Cancer Testing of Protein Molecules and Particularly Growth Factors

J. B. Clarke, N. Dybdal and A. Cheng,
Genentech, Inc. South San Francisco, CA, USA

Three studies in both p53 (het) and Tg.AC mice were designed to address false positives caused by injection or an immune response to the protein, and the response to chronically administered growth factors. In study 1, animals received either no injection, saline, pH 5.5 vehicle or human albumin (1 mg/kg). In study 2 and 3, murine epidermal growth factor (mEGF) at 0.1, 1.0 or 10 µg/kg or recombinant human insulin-like growth factor-1 (rhIGH-1) at 0.1, 1.0 or 10 mg/kg were administered respectively. The p53 mice used in study 1 were bred at Genentech Inc., remaining animals were from Taconic. In each study, treatments were administered 3 times per week, subcutaneously for 6 months, clinical pathology and hematology were performed prior to and at 3 and 6 months and animals were necropsied at 6 months for subsequent microscopic pathology. Blood samples were also drawn after 3 months to confirm exposure to the growth factors. All Tg.AC mice were tested retrospectively for the responder phenotype by Taconic. No apparent growth factor treatment-related gross lesions were observed. Unfortunately, injection and immune-related tumor formation in the Tg.AC model (study 1) could not be evaluated due to a 16% overall responder phenotype in this study. In study 2 and 3 the overall Tg.AC responder rate was 50% males/0% females and 83% male/91% female respectively. In studies 2 and 3, after 10 weeks, a small number of male Tg.AC mice developed small botryoid perineal masses which did not appear to be treatment related. Also in studies 2 and 3, a few p53 mice developed subcutaneous masses consistent with areas of probable prior irritation at the injection site. In conclusion, neither model may be sensitive to an increased tumor risk as a result of chronic administration of either mEGF or rhIGF-1. Alternatively, this may indicate that chronic administration of growth factors is not associated with an increase in tumor risk, particularly since the Tg.AC model, which would be expected to respond to potential nongenotoxic carcinogens, was not responsive to either growth factor.

Contact J.B. Clarke for copies of the poster presented on this topic at SOT 99
(650) 225-3943
clarke.janet@gene.com

Upcoming Meetings and Events

☺ 9th North American ISSX Meeting
Opryland Hotel, Nashville, TN

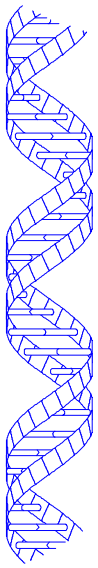
October 24-28, 1999

☺ Establishing & Maintaining Rodent Production Colonies
Andover Marriott, MA (Host: Charles River Labs)

June 20, 1999

☺ EUROTOX 1999
Oslo, Norway

June 27-30, 1999



A B C D

S. Furst, RD 9-5
Research & Development Center
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